

BACTERICIDAL ACTION OF WATER TREATED BY ULTRA VIOLET RAYS

W. F. WALKER, *Deputy Commissioner of Health, City Department of Health,*
and R. W. PRYER, D. P. H., *Director of Laboratories,*
Detroit, Mich.

Ultra violet ray sterilization of water acts in an unusual manner. Exposure to the rays not only kills bacteria then present in the water, but more than this, there is imparted to the water a potential sterilizing power which kills bacteria subsequently added to the water.

HISTORICAL

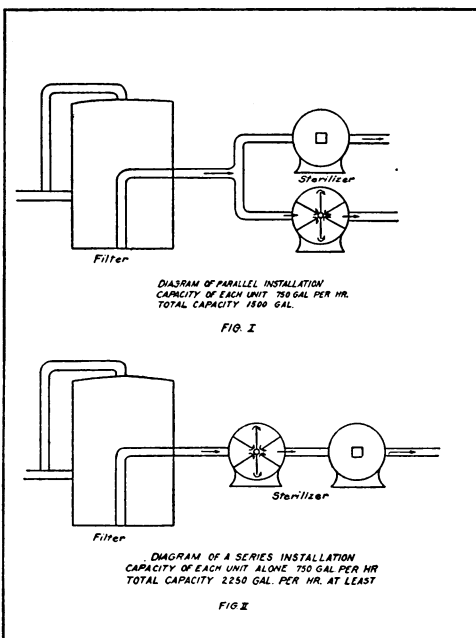
THE bactericidal action of light was first pointed out by Downes and Blunt in 1877 in a paper before the Royal Society of London. They called attention to the fact that direct and even diffused sunlight has the power of killing the bacteria of putrefaction. The most active rays are the blue, violet, and ultra violet frequencies, although the red and orange are not entirely inactive. Heat rays, however, play no part in this action.

In 1892 Professor Marshall Ward with Sir Oliver Lodge exposed culture plates to the ultra violet energy of the electric arc alone as well as to the energy of other parts of the spectrum, establishing a much more powerful bactericidal action with the ultra violet light alone.

The mechanism of this bactericidal action has been investigated by W. E. Burge of the University of Illinois, who has presented evidence to show that ultra violet radiation kills living cells by coagulating their protoplasm. That this action occurs most vigorously in the first instant of exposure is shown by Parkinson in the report of the Provincial Board of Health of Ontario in 1914. His experiment in the sterilization of water and sewage indicates that the number of organisms surviving does not bear a constant relation to the number in the influent. In fact, the result seems to be

as low with several thousand organisms as with a few hundred in the influent.

Where large quantities of water are to be treated it has been the practice for some time to use series installations of ultra violet ray sterilizers. It has been



shown by practical application that with two sterilizers of say 750 gallons per hour capacity, when connected in parallel as shown in Fig. 1, the ultimate capacity for safe treatment is 1,500 gallons per

hour. However, if these same sterilizers are connected in series as shown in Fig. II the ultimate capacity is not 1,500 gallons per hour, but is at least 2,250 gallons per hour, or more, depending to some extent on the quality of the water. If three sterilizers are placed in series a second increase in capacity of about 50 per cent is obtained.

These results lead to the conclusion that the bactericidal effect of ultra violet ray treatment does not lie alone in the direct exposure of all the organisms to the rays.

DEVELOPMENT OF RADIO-ACTIVITY IN THE WATER

A possible causative factor for the increased capacity of series over parallel installation would be the development of radio-activity in the water due to exposure to the ultra violet rays, which would exert a bactericidal effect after the water had passed beyond the range of the direct rays of the lamp. It was to investigate this possibility that these studies were made. Kreusler has demonstrated that the rays in the neighborhood of 1,860 *angstrom unit, are about 69 per cent absorbed by two centimeters of water, and that this absorption decreases with an increase of wave lengths. †Lyman states that 5. mm. of water give 100 per cent absorption of rays of wave lengths equal to 1,729 angstrom units, or less.

The quartz mercury vapor arc such as is used in the commercial type of sterilizer is particularly rich in rays of a length equal to 1,849 angstrom units, which have been shown to be particularly bactericidal. It is pointed out that in the layer of water which surrounds the lamp all of the rays of high bactericidal power are absorbed by the medium. We know from the work of the early investigators that it is the rays which are absorbed that are photochemically active.

*One angstrom unit equals one ten-millionth of one centimeter.

†Lyman, Spectroscopy of Extreme Ultra Violet Rays.

DESCRIPTION OF DETROIT EXPERIMENT

The first work was done on samples taken from the water purification plant of the Detroit & Cleveland Navigation Company boats. The installations on the steamers City of Detroit III, and the City of Cleveland III, were of the series type and sampling points were established after filtration and after sterilization, so that samples of filtered water without sterilization, and samples of filtered and sterilized water were obtained. These will be known throughout as sample points A and B respectively, A denoting filtered water, and B filtered and sterilized water.

Ten-ounce samples were collected after the sterilizer had been running at least fifteen minutes, marked with a number, and data as to their source recorded. No effort was put forth to hurry these to the laboratory. The other daily samples were collected and all taken in at one time, usually about 10:30 or 11 a. m. On arriving at the laboratory these samples were run at the same time as other water samples, usually about the middle of the afternoon, which resulted in a total holding of the samples of from three to four hours from the time of sterilization until the laboratory work was done.

LABORATORY PROCEDURE

Both sample A and B were plated in duplicate on ordinary water agars with a pH reading of 7, both 1 cc. and 0.1 cc. dilutions being used.

To a 10 cc. portion of what remained of each sample there was added one drop of a suspension of organisms made up as follows: A standard loop of a fresh 24-hour culture of a Para B typhoid or some resistant spore bearing organism was added to 10 cc. of normal sterile salt solution and agitated to break up clumps of bacteria. One drop of this suspension was transferred by a 1 cc. pipette to another tube containing 10 cc. of normal sterile salt solution and again agitated. One drop of this second suspension was added by a 1 cc. pipette to the 10 cc.

portion of each of the samples, and 1 cc. and 0.1 cc. plates were poured from them. The remainder of each sample was kept at room temperature. At the end of one and two hours respectively plates of these samples were again made in the same dilution. All plates were incubated at 37° C. for 24 hours. The media used was prepared according to the standard methods of water analysis as prescribed by the American Public Health Association in 1919, and the plates were counted in the usual manner, using a hand lens.

Table I shows the average results of a series of seven samples, using a spore bearing organism for inoculating to the samples.

TABLE I

Source	Original count	Count after adding equal volumes of suspension of spores	Count at 1 hr.	Count at 2 hr.
Filtered water sample Pt. A.	612	1928	2243	2360
Filtered and sterilized water sample Pt. B.	7	77	107	60
Average of seven series.				

The real significance is somewhat clouded by the original count. To make the result clear Table II is prepared, showing the number of organisms added and living at the 1 and 2-hour periods. These figures are obtained by subtracting the original count from the results given in Table I.

TABLE II

Source	Organisms added and living	Count at 1 hour	Count at 2 hours
Filtered water sample Pt. A	1316	1631	1748
Filtered and sterilized water sample Pt. B.	70	100	53

The laboratory work was carefully controlled so that the technician did not know until after the plates were counted which samples were sterilized and which were not. There is no reason to doubt but that the same number of organisms was added to each sample, yet it is clearly evident that only about 6 per cent survived the exposure to the treated water, and that these tend to die out rather than to multiply. It has been stated by *Fair and others that spores, because of their

greater transparency, are more readily killed by ultra violet light than other vegetative forms. However, Para B typhoid shows a remarkably high susceptibility to the influence of treated water. Table III shows the average results of six series of samples using suspensions of Para B typhoid for inoculation purposes.

TABLE III

Source	Original count	Count after adding equal volumes of suspension of Para B typhoid	Count at 1 hr.	Count at 2 hrs.
Filtered water sample Pt. A.	1037	1160	1200	1730
Filtered and sterilized water sample Pt. B.	5	21	51	97

Table IV shows the results expressed in Table III after subtraction of the original count.

TABLE IV

Source	Organisms added and living	Count at 1 hour	Count at 2 hours
Filtered water sample Pt. A	123	163	693
Filtered and sterilized water sample Pt. B.	16	46	92

Tables V and VI show the results of a series of 42 samples using a suspension of Para B typhoid for inoculation. In this series the water used was collected from the treatment plant in connection with swimming pools of this city. Sample A was collected from a pool having filters only, and Sample B from a pool where the treatment was by filters and ultra violet ray sterilizers, namely, at the Detroit Athletic Club. It is of interest to note in this connection that the results of filtration in each instance were very uniform, and that at the time this work was done only one of the sterilizers at the athletic club was in operation, which accounts for the fact that the count is only slightly lower than that of the filtered water from the other pool. The bactericidal action of the treated water, however, is very clearly shown. In spite of the fact that the counts are less than one hundred apart originally, after addition of the organisms, the count for the filtered water climbs rapidly, while the treated water falls to a figure below its original count.

*Gordon M. Fair. "Disinfection by Ultra Violet Light." Journal American Water Works Association. May, 1920.

TABLE V

Source	Original count	Count after adding equal volumes of suspensions of Para B typhoid	Count at end of 1 hr.
Filtered water sample Pt. A	472	583	686
Filtered and sterilized water sample Pt. B..	378	388	233

TABLE VI

Source	Organisms added and living	Count at 1 hr.
Filtered water sample Pt. A	111	164
Filtered and sterilized water sample Pt. B.	10	0

Through the courtesy of the James B. Clow Company of Chicago the authors were enabled to work with a small experimental plant having a filter and two sterilizers of the gravity type operating on a 110-volt circuit. Considerable experimentation with this equipment under normal working conditions of flow failed to show that any bactericidal property was imparted to the water. However, later experiments with samples exposed in petri dishes, or crucibles, for a period longer than that of normal flow showed a very pronounced bactericidal action in every instance.

In this work samples of tap water which were boiled and cooled to render them sterile and of distilled water were exposed to the action of ultra violet light from the lamp of one of the gravity type sterilizers. The average results are expressed in Table VII.

TABLE VII

Source	Original count	Count after adding suspensions of Para B typhoid	Count at 1 hr.	Count at 2 hrs.
Tap water boiled not exposed..	0	767	695	272
Tap water boiled, exposed to U. V. R. 6 min..	0	637	389 Count after 20 min.	0 Count at 6 hrs.
Distilled water not exposed..	0	716	540	270
Distilled water exposed to U. V. R. 6 min.	0	80	3	0

In these later studies there is no initial count to confuse the results, and it is clearly apparent that under these unusual conditions of sterile water to be treated, the added organisms die out rather rapidly. However, those in the treated water undergo a more rapid reduction. The same laboratory technique was employed throughout.

It seems significant from this work that the bactericidal property is dependent upon the energy input into the water, that is, a high voltage for a short exposure, or a low voltage and longer exposure will accomplish the same result. A number of impulses of short duration at a high voltage seem, however, to accomplish greater bactericidal action than a longer exposure and a lower voltage, and of one exposure only.

CONCLUSION

It seems quite reasonable to the writers therefore to conclude:

1. That the exposure of water to ultra violet light emitted from a quartz mercury vapor arc, imparts to the water a definite residual bactericidal property, which effects a reduction of at least 85 per cent of the organisms subsequently added.

2. That the ability to impart this property is dependent upon the energy input into the lamp, and the time of exposure of the water.

3. That the usual pressure type of sterilizer operating on a 220-volt circuit develops the bactericidal property under ordinary working conditions.

4. That this property is accentuated in series installations.



HEALTH INSTITUTE

The next issue of the News Letter will describe the Health Institute to be held in New York City one week before the Annual Meeting. Watch for the News Letter!